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Wnt signaling molecules have been implicated in mouse mammary carcinogenesis. β-catenin, a downstream molecule in the Wnt pathway, activates transcription of target genes. Our studies are aimed at determining whether β-catenin can function as an oncogene in the mammary gland. Using the mouse as a model system, we targeted expression of β-catenin to the mammary gland in transgenic animals. Preliminary results have revealed that β-catenin transgenic lines succumb to mammary tumors characterized as microacinar adenocarcinomas at a mean age of onset of 6 months. Transgenic lines expressing a dominant negative form of β-catenin in the mammary gland have also been established. These mice are being bred to Wnt-1 transgenic lines, which normally get tumors at 3-6 months of age, in an attempt to delay the onset of tumorigenesis. We are also interested in identifying downstream transcriptional targets of β-catenin in the mammary gland. To that end, attempts to express an inducible form of β-catenin in a mammary epithelial cell line are in progress. We have also established tumor cell lines from the β-catenin tumors which will be helpful in identifying downstream targets of β-catenin. Thus, our studies have begun to establish β-catenin as an oncogene in the mammary gland and aim to identify β-catenin targets in β-catenin-mediated mammary oncogenesis.

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INTRODUCTION

Wnt signaling molecules are capable of inducing mammary tumorigenesis in the mouse. β-catenin functions in the Wnt signal transduction cascade as a transcription factor which, in conjunction with Tcf/Lef factors, can activate downstream target genes. We are interested in investigating the putative role of β-catenin as an oncogene in the mammary gland, using the mouse as a model system. In particular, our studies aim to evaluate the tumorigenicity of β-catenin, identify the downstream targets of β-catenin in the mammary gland, and correlate β-catenin with the tumorigenic capability of Wnt molecules. These studies are of particular relevance given a recent report demonstrating a potential link between between β-catenin upregulation and human breast cancer.

ANNUAL SUMMARY

Given that the Wnt pathway has been implicated in breast cancer, we were interested in assessing whether β-catenin, which functions downstream in the Wnt signaling pathway, may play a role in mammary tumorigenesis. In an attempt to test whether β-catenin can function as an oncogene in the mammary gland, the mouse was chosen as a model system and a transgenic approach was taken.

An MMTV-promoter was used to target expression of a constitutively active form of β-catenin, lacking the 90 amino-terminal amino acids of the protein (ΔN), to the mammary gland. Four transgenic lines were generated from founder animals, although one line was eliminated due to lack of transgene expression in the pregnant mammary gland. Line ΔN-9281, expressing the highest levels of transgene expression, has been most carefully followed. To date, seven female mice have succumbed to mammary tumors at a mean age of onset of approximately six months. Six of these mice were continuous breeders, having undergone 2-6 rounds of pregnancy, while the seventh was a virgin. Several of the mice were found to have multiple primary mammary tumors. Histopathologic analysis has demonstrated the majority of the tumors to fall into the category of microacinar adenocarcinomas. No obvious metastases have been observed. Whole mount analysis of (tumor-free)mammary glands from 9281 females has revealed regions of hyperplasia and potentially microscopic tumor foci. In an additional transgenic line, namely line ΔN-9266, the founder, a female (multiparous-4) came down with a mammary tumor at 10 months of age. In the third β-catenin-expressing transgenic line, ΔN-9801, two animals have succumbed to mammary tumors, including a 12-month old male. All transgenic lines are being continuously monitored for mammary tumors on a weekly basis.

Genetic collaboration in mammary oncogenesis, whereby two oncogenes together vastly accelerate the onset of tumorigenesis, has been observed in several instances. In particular, in mice bearing both MMTV-Wnt-1 and MMTV-Int-2

(Fgf3) transgenes, onset of tumors is significantly more rapid as compared to mice harboring only a single transgene. Given β-catenin's role in the Wnt pathway, we are interested in testing whether β-catenin can collaborate with Fgf3 in accelerating tumorigenesis. Double transgenics, carrying both constitutively active β-catenin and Fgf3 transgenes, are currently being generated and will be monitored for appearance of tumors. An accelerated tumorigenic phenotype is expected.

The next set of experiments attempt to confirm the role of β-catenin in the Wnt pathway, and specifically the role of β-catenin in effecting Wnt-mediated mammary tumorigenesis by means of transcriptional activation. A dominant negative form of β-catenin mice was generated, whereby the carboxy-terminal putative transactivation domain, likely to be required for activation of downstream targets, was deleted. An MMTV-promoter was used to drive expression of the carboxy-terminal truncated form of β-catenin (ΔC) to the mammary gland. Three β-catenin dominant negative transgenic lines were generated, all of which express the transgene in the pregnant mammary gland. The highest expressing transgenic line, ΔC-3035 was chosen to be bred with MMTV-Wnt-1 transgenic mice. Double transgenic mice are currently being generated and will be monitored for appearance of mammary tumors. The expectation is that Wnt-1 mediated mammary tumorigenesis will be halted or at least delayed due to the presence of a dominant negative β-catenin molecule.

In an attempt to enable further dissection of the molecular pathways involved in β-catenin-mediated mammary tumorigenesis, tumor cell lines are being generated from the β-catenin-ΔN mammary tumors. To date, two tumor cell lines have been generated, each of which expresses high levels of the transgene, as monitored by both Northern and Western blot analysis. Furthermore, expression levels of the transgene in both cell lines is dexamethasone inducible, as expected for an MMTV driven construct. In transient transfection assays using a Tcf/Lef

reporter construct, β-catenin was demonstrated to be transcriptionally active, again in a dexamethasone inducible fashion. Additional β-catenin-ΔN tumor cell lines, as well as Wnt-1 and Wnt-10b cell lines, are currently being established. A panel of β-catenin and Wnt tumor cell lines will ultimately be important in identifying and verifying downstream transcriptional targets of β-catenin in the transformed mammary gland.

To aid in the identification of downstream targets of β-catenin, an inducible expression system for β-catenin in a mammary epithelial cell line is currently being established. Both β-cateninΔN and β-catenin-ΔC have been placed under a promoter that requires tTA for expression. These constructs have been introduced by stable transfection into a clone of the mammary epithelial cell line Eph4 expressing tTA in a tet-inducible fashion. To date, two clones have been isolated that express β-catenin-ΔC in a tet-dependent manner. Isolation of β-catenin-ΔC expressing clones is currently in progress. The β-catenin inducible expressing cell lines will be used in a genome-wide screen to identify target genes that are up-regulated in response to induced β-catenin expression (β-catenin-ΔN-expressing clones) and potentially downregulated in response to induced expression of dominant negative β-catenin (β-catenin-ΔC-expressing clones). Ultimately, the β-catenin-derived tumor cell lines can be used to verify any targets identified in this screen.

The β-catenin studies outlined here are, and will continue to be, valuable in helping to elucidate this novel model of mammary tumorigenesis.

Key Research Accomplishments

- Established MMTV-driven constitutively active β-catenin transgenic mouse lines

- Found tumors (predominantly microacinar adenocarcinoma) at mean age of onset = 6 months of age in mice expressing β-catenin transgene

- Established MMTV-driven dominant negative β-catenin transgenic mouse lines

- Established mammary tumor cell lines derived from β-catenin murine mammary tumors

- Established mammary cell line with inducible expression of dominant negative β-catenin

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